III.
2 f Kidney and conveying Urinary Organs

In the postulated correlation between inhalation of cigarette smoke-components and tumor formation in the bladder - literature see SCHIEVELBEIN and ZICKGRAF (1968) - a more exact examination of the mucous membrane of the bladder seemed necessary in the test animals. Since the animals had absorbed a high concentration of smoke-components, a tumor-triggering effect in the bladder was within the range of possibilities.

The evaluation of the findings of the histological examination of the bladder showed, besides sporadically occuring inflammable diseases of the bladder wall and the mucous membrane (a total of three cases of severe bladder inflammations in groups 6, 8 and I), only in one animal of group I8 A (nitrosamin without smoke-treatment) a typical, mildly papillary carcinoma of the bladder (ill. 43) with beginning wall infiltration.

Spontaneous bladder carcinomas have so far not been described in the hamster. Through treatment with o-aminoazotoluene, it was possible to produce bladder carcinomas in a frequency resting far higher than in other test animals (TOMATIS and Assoc. 1961).

Tumors of the kidney were also extremely rare in our stock. We found in one treated animal (group I8 - smoke-treatment - died before the additional treatment with nitrosamin) one renal pelvis carcinoma (ill. 44) with, in parts, papillary structure of the distinctly infiltratively growing transitional epithelium.

Spontaneous kidney tumors had been sporadically observed already earlier by FORTNER and GALE (1958), FORTNER (1957, 1958), DUNHAM and HARROLD (1962), FORTNER (1961), TOMAN (1963), BODIAN and RIGBY (1964), TOTH (1967). They were cases of adenocarcinoma and of nephroblastomas (Wilm's Tumor). That different carcinogens are tumor-producing in the kidney of the goldhamster (nitrosamins - TOMATIS and Assoc. 1964; benzpyrene - DONTENWILL and RANZ (1960) was proven by the mentioned authors.

Besides the described tumors and the diseases of the kidney, discussed under amyloidosis, a small number of inflammable kidney diseases (nephritis, pyelonephritis) were found independent of the test arrangement (chart 66). We should also mention the sporadic occurances of kidney cysts (2 cases in group K, one case each in group I and

group I0), (see cysts of the liver).

A comparison between kidney weight and heart weight seemed significant to us, particularly in view of the appearance of "arteriosklerotic" or inflammable vascular diseases, respectively the formation of renal high pressure. STROLA and Assoc. (I954) had proven that a renal ischemia in the hamster leads to a rise in blood-pressure.

In the charts 67 and 68 are compiled the average heart and kidney weights of the different test groups for the stages I, 2 and 3 of kidney amyloidosis, see chapter "Generalised Amyloidosis". It is conspicuous that the average kidney weight of the animals with an amyloidosis-stage 2 is higher in all groups than the corresponding weight for the total number of the animals; the kidney weight of the animals with amyloidosis-stage 3 however, is in most groups lower. It was possible to ascertain the differences between stage 2 and stage 3 with overwhelming statistical evidence (with a probability of error of 5 %). In the females of group 5 as well as in the males of the groups 3 and 6 , a difference in kidney weight between stage 2 and 3 could not be ascertained statistically.

The heart weights on the other hand did not show any distinct dependency on the amyloidosis-stage of the kidney. In most groups the average heart weight in stage 3 rests under that of stage 2. But the differences are mostly not significant. Only in group 6 ♂, the difference between stage 3 and stage 2 is statistically ascertained. In several groups (K ♂, I ♀, 2 ♀, 4 ♂ 5 ♀ and 9 ♂), the weight is higher in stage 3 than in stage 2, however this increase could not be ascertained.

For a further analysis of this correlation, we investigated the correlation between difference in body-weight and kidney-amyloidosis. In the charts 69 and 70 are listed the average weight differences (between end-weight and initial weight) for the animals with the different amyloidosis-stages. Almost all groups show here a distinct negative dependency of the weight difference and thus of the end-weight from the amyloidosis-

stage; that is, the end-weight of the animals is considerably lower in stage 3 than in stage 2 or I. Particularly great (and highly significant) is the difference in the groups K, 3, 4 and 5. In the smoke-exposed group 6 on the other hand, the difference between stage 2 and stage 3 is only minor and statistically not significant. The influence of the kidney-amyloidosis on the weight of the animal is here obviously covered over by the influence of the smoke-treatment.

The dependency of the end-weight from the amyloidosis-stage suggests to calculate and examine not only the absolute heart weight but also the relative heart weight (relative to 100 g body-weight)for the three amyloidosis-stages. These relative heart weights for the different groups and stages are listed in the charts 71 and 72. It becomes evident from these charts that these relative heart weights in the groups K, I and 2 as well as 5 are distinctly higher in stage 3 than in stage 2 or stage I. In group 3, the relative heart weight is higher only in the males. In group 6 on the other hand, in males and females, the relative heart weight in stage 3 is distincly lower than in stage 2 and (only in the males) in stage I. In the remaining groups there is not distinct difference in the relative heart weight between the amyloidosis-stages.

III.
2 g Generalized Amyloidosis

In earlier research on the amyloid formation in the goldhamster, (DONTENWILL and Assoc. I960) we had reported on the frequent appearance of amyloidosis (up to I00 %) particularly in the old age of the animals. These findings have meanwhile been confirmed by GLEISER and Assoc. (I97I) and BLAHA (I967) and others. We had termed this form of the amyloidosis as a primary amyloidosis and had compared the genesis of the amyloidosis disease to the primary amyloidosis in man.

While we did not undertake a division in stages in the evaluation of the amyloidosis of the spleen, the liver and the suprarenal gland, despite considerable differences in the degree of amyloid deposists, we did distinguish between three stages of severity (chart 73) in the kidney-amyloidosis, particularly in view of possible effects on the heart and the vascular system (see under Kidney and Cardiovascular System):

I. a moderate to intermediate amyloid deposit (ill. 45 a) in the area of the glomerulus loops, and an amyloidosis of Bowman's Capsule with only a minor nephrotic component,

2. a strong amyloidosis (ill. 45 b) of the glomeruli and the basal membranes of the tubuli as well as the interstitial vessels with considerable amyloid-nephrosis,

3. an extreme-severe amyloidosis (ill. 45 c) of the kidney with vast shrinking of the kidney parenchyma, a strong nephrotic component and increasing lack of functioning of the nephrons.

A predominance of the female animals became evident in stage 2, as it had been for the liver, spleen and suprarenal gland. The difference between males and females was statistically examined with an X^2- test, and the differences were very highly significant (X^2= I06.2 with I FG). An influence of the smoke-treatment was not recognizable, not even in the male animals with a longer survival period. The differences between the groups 3, 4, 5 and 6 as well as 8 were statistically only weakly ascertained with an

X^2- test ($X^2 = 10.7$ with 4 FG, ($P < 5\ \%$)). However, the differences between these group were highly significant for the females ($X^2 = 32.5$, ($P < 1\ \%$)). A difference in frequency of the same extent was not provable between male and female animals in stage 3. ($X^2 = 3.56$, ($P > 5\ \%$)). A higher frequency was evident here only in individual smoke-exposed groups. In the females as well as in the males, the differences between the groups 3, 4, 5, 6 and 8 were highly significant ($P < 1\ \%$).

The amyloidosis of the kidney was frequently accompanied by strongly homogenous calcareous deposits in the tubuli clearances, respectively the collecting ducts.

The amyloid deposits of the liver (ill. 46) were as a rule most strongly provable in the periportal fields, as described already earlier. The greater frequency in female animals, despite a lower average age, is conspicuous in the amyloidosis of the liver as well as of the spleen (chart 74). The differences between males and females were in this case very highly significant ($X^2 = 114.7$ with 1 FG). On the other hand, the differences between the smoke-exposed groups 4, 5 and 6 and control group 3, respectively placebo group 8 were highly significant only for the females while they could not be ascertained for the males.

The amyloidosis of the spleen was predominantly a pulpa-amyloidosis. Similar to the liver, the differences between the two sexes were also highly significant in the amyloidosis frequency of the spleen. The differences between the smoke-exposed groups and the control group, respectively the placebo group were only in the females highly significant ($X^2 = 37.0$ with 4 FG) while they could not be ascertained for the males ($X^2 = 6.81$).

The amyloidosis of the suprarenal gland (ill. 47) often led to an extreme atrophy, as already described earlier. Here too, a greater frequency was evident in the female animals.

In the evaluation of the frequency distribution of the amyloidosis, we gave special

consideration to the increase of the degree of severity with rising age in relation to the treatment of the test animals and to traceable inflammable changes of the organs and the tissue. We obtained neither unequivocal correlations to inflammable changes nor an unequivocal influence of the treatment on the frequency and degree of severity of the amyloidosis. The obtained findings once more confirm the opinion voiced already earlier, that the amyloidosis is a case of a hereditary spontaneous disease of the hamster which attacks over 90 % of the animals with increasing age.

In the charts 75 and 76 are listed for the different test groups and separated by sex the number and the percentage rate of animals which in the pertaining age groups (0 - 25, 26 - 50, 5I - 75, 76 - I00, $\geq$ I00 weeks) showed the stages I, 2 or 3 of a kidney amyloidosis.

The charts show (75 and 76) that the frequency and the degree of severity of the kidney amyloidosis depend unequivocally on the age. This becomes particularly evident in the frequency of stage 2 in the females which mostly rises from 0 % in the age group 0 - 25 weeks to 80 % up to I00 % in the age groups over 75 weeks. Moreover, the stages 2 and 3 are found predominantly in the higher age groups while stage I often appears only in the age groups under 75 weeks in the females. In the males on the other hand, stage I also occurs in the higher age groups (over 75 weeks) while it was practically not observed in the age groups from 0 - 25 weeks.

Altogether, the charts 75 and 76 show distinct differences between males and females which can not be explained through the different age distribution. Instead, the amyloidosis is generally less frequent in the males; it can be observed predominantly in the higher age groups and does indeed in its frequency show an age dependency but not in a degree of severity as does the kidney amyloidosis. All three severity stages occur in the age groups over 50 weeks with approximately the same frequency.

In the females however, we obtain a distinct dependency of the frequency and the degree

of severity on the survival period.

An influence of the smoke-treatment on the amyloidosis frequency could not be ascertained, neither in the males nor in the females. Particularly the amyloidosis stage 2 is indeed the lowest in group 6 for males as well as females; but this effect may be attributable to the fact that in group 6 the average survival period was the shortest and that thus the animals did not reach an age sufficient for the formation of an amyloidosis.

We also found anoften severe vascular amyloidosis (ill. 48) in other organs like the pancreas, the thyroid gland and the testicles (see Gonads), respectively a para-amyloidosis which we did not evaluate quantitatively.

The stages of the amyloidosis are essentially correlated with the survival period and show no correlation to the smoke-treatment.

giant cells (COHRS and Assoc. 1958). Spermiocytes and giant spermid cells of the testicle tubules also occured in a largely intact spermiogenesis and were observed relatively rarely in extremely atrophic testicles, consequently they are not at all a regular finding in atrophy, to some extent, the atrophic testicles show patterns as they had been described by GIESE and HORSTEBROCK (1962) for quantitative lack of nourishment in man.

The evaluation of the findings of the testicles (chart 77) demonstrates that in all test groups, a, in parts, considerable atrophy becomes evident with increasing age, which is demonstrated by the reduction of the testicle weight as well as in the histological finding "atrophy". The critical value obviously rests at a testicle weight of circa 0.7 g. Almost all testicles below this weight mostly show an advanced atrophy. The average values of the relative testicle weights (relative to 100 g animal weight) have already been listed in the chart of relative organ weights. For a statistical evaluation of the testicle findings, we calculated for each group the average survival period and the average testicle weight as well as the maximal testicle weight for the animals with testicle atrophy. The results are compiled in charts 78 - 80. For the purpose of a comparison, we also listed the total average values for the survival period and the testicle weight of all animals of the groups in question in the chart.

It becomes evident, that the average survival period for the animals with testicle atrophy is in all groups considerably higher than that for all animals of the group in question. The difference amounts to up to 64 % of the total average (in group 16). A systematic influence of the smoke-treatment can not be established from the results of the chart. The average survival period is the longest in control group 3 for all animals as well as the animals with testicle atrophy. While however, in all animals of the smoke-exposed group 6, the survival period drops significantly, a similarly significant drop for the animals with testicle atrophy can not be traced in group 6.

It is remarkable that the frequency of testicle atrophy in group 6 with 3I % is practically just as low as in group K with 32 %. Consequently, an influence of the smoke-treatment is also not traceable in the testicle atrophy. The chart shows furthermore that the average testicle weight in the animals with testicle atrophy rested considerably below the average testicle weight of all animals. It is particularly low in group 6, eventhough the total average is relatively high in this group. However, even in group 8, differences in the average testicle weight between the atrophy-animals and the remaining animals are still relatively great. Therefore, these differences cannot be attributed to the smoke-treatment. It is furthermore remarkable that, in control group 3, the testicle weight of the atrophy-animals is the highest with 0.4 g.

In chart 80 is compiled the frequency of testicle atrophy for the 5 different age groups (0-25, 26-50, 5I-75, 76-I00, >I00 weeks). For each age group and each group are listed the number n of the animals of this age group, the number n of the animals with testicle atrophy in the age group in question, and the percentage rate of animals with testicle atrophy in the age group in question. It becomes evident that this percentage rate definitely rises in each group with increasing age. While in the age group from 0-25 weeks not one single animal shows a testicle atrophy, more than half of the animals have testicle atrophies in the two highest age groups in almost all the test groups. The frequencies in the highest age groups (> I00 weeks) demonstrate greater fluctuations because of the small numbers of animals in the group. The age dependency of the testicle atrophy does obviously not depend on the treatment. However, the frequency of testicle atrophy varies in the different groups. An influence of the smoke-treatment is not recognizable.

In order to be able to determine more precisely the influence of the smoke-treatment on the relative testicle weight, we established the absolute and relative testicle weights in different test groups which were killed after a test length of I3 weeks. As chart 8I shows, it is thereby unequivocally evident, that through the remaining of the animals in the smoke tubes of the smoking apparatus without smoke-supply,

that is, with the same length of remaining in the smoke tubes and a supply of fresh air, the testicle weight is considerably influenced. While the average testicle weight of the control animals (with same age, same test length and keeping)without any treatment amounted to 3.23 g per I00 g body-weight, the animals treated in the apparatus with fresh air showed a strong reduction of the testicle weight. The reduction from 3.23 g to 0.72 g per I00 g body-weight is the equivalent of a reduction of 3/4 in comparison to the control animals. According to the experiences of other researchers, the testicle of the hamster is obviously very sensitive to outside influences. Whether the reduction of the testicle weight is caused by a shifting of the testicles into the abdominal cavity during the exposure to fresh air could not be determined. When one compares the test animals treated with fresh air to the smoke-exposed test animals, one finds in the smoke-exposed animals a reduction of the average testicle weight from 0.72 g to 0.47 g per I00 g body-weight. This difference is significant ($p \leq 0.00I$). However, the reduction is vastly smaller than in the comparison between the untreated and fresh-air controls (3.4 g to 0.72 g per I00 g body-weight values of the inidividual groups - see chart 8I). The present test results demonstrate that the hamster is obviously not a suitable test object on which to measure, in the present test arrangement, the influence of smoke-substances on the testicle weight, respectively the testicle function. The in itself already strong fluctuation of the testicle weight in the hamster complicates a comparative observation even without treatment, particularly in older animals. In the critical assessment of the total findings, an unequivocal effect of the smoke-substances on the testicle function is not ascertainable.

An extraordinary thickening of central vessels of the testicles was in some parts found in individual animals. Theses thickened vessels show a positive amyloid-reaction, in parts, the lumen of the vessels is hardly recognizable at all. Similar changes were described by GLEISER and Assoc. (I97I). The frequency of the vascular amyloidosis fluctuates very strongly, it is the highest in the animals treated only with asbestos

or gas-phase. An influence of the smoke-treatment is not ascertainable.

Besides the described changes, there were individual cases of inflammable processes in the testicles and ossifications in the area of old inflammation foci.

Testicle tumors were not provable. Prostata tumors were also not observed.

Only sporadic spontaneous tumors in the genitals of the male hamster have so far been described by other authors. (FORTNER (I96I) described among 94 hamsters 2 adenocarcinomas of the prostata, KIRKLAN (I962) I seminoma as well as 9 (testicle and globus minor) adenomas among 7200 hamsters, FORTNER and Assoc. (I963) I adenocarcinoma of the prostata.

In the ovaries , we found sporadically variedly-sized smooth-walled cysts, lined with regular epithelium (chart 77). In group I (DMBA and smoke) 20 % of the animals had cysts in the ovaries, in group 2 (DMBA) even 30 %. In control group 3 on the other hand, only 6 % of the animals had cysts in the ovaries. The difference between the groups treated with DMBA and the control animals is highly significant ($X^2 =$ I3.I2 with 2 FG, ($P <$ I %)). It is probable that DMBA damages the follicles which then degenerate into cysts,as KRARUP (I969) was able to demonstrate in mice. The smoke-exposed animals do not show a varying development of the follicle in the ovaries in comparison to the controls.

In the area of the female genitals, inflammable changes could be proven only in individual animals which, however, did not show a correlation to the nature of the treatment.

The observed tumors did also not demonstrate an increased frequency in the treated test groups as compared to the controls.

Among tumors must be mentioned benign papillomas of the mucous membrane of the corpus and the cervix (ill. 50) as well as hemangiomas of the mucous membrane of the corpus (chart 82).

FORTNER (I96I) also observed similar tumors. TOTH (I967) found I adenocarcinoma among I00 hamsters.

Sporadically, we found small, easily delimitable leiomyomas of the corpus uteri (ill. 5) whose structure is vastly similar to the tumors described in man.

Individual cases of similar tumors were described already earlier by FORTNER (I957, I96I), FITE (I958), FORTNER and GALE (I958). TOTH (I967) describes I leiomyosarcoma among I00 hamsters.

Among the carcinomas of the uterus (chart 82), we found two types. Exophytically growing papillary corpus carcinomas and strongly infiltratively growing, more endophytically growing, adenocarcinomas of the mucous membrane of the corpus uteri (ill. 52).

Similar tumors had been proven already earlier by LINDT (I958), FORTNER and GALE (I9[illegible]) FORTNER (I958) (9 among 620 hamsters), FORTNER (I96I) (5 among 87 hamsters), KIRKMAN (I962) (3 among 7200 hamsters) and DUNHAM and HERROLD (I962) (3 among 360 hamsters). Only SHERMAN and Assoc. (I963) had reported of an adenocarcinoma of the cervix.

Ovarian tumors are rare in the hamster. We found only 3 small luteomas (ill. 53) and and I granulosa cell tumor with characteristic structure.

They were also described in similar form by FORTNER (I957, I96I) and KIRKMAN (I962) (2 among 7200 hamsters). Malignant ovary tumors were not provable. Other authors have also described individual cases of granulosa cell tumors (2 among 360 hamsters), (DUNHAM and HERROLD, I962)

The frequency of the tumors was so small that a statistical test does not make sense. It is however remarkable, that of the 4 observed benign tumors of the ovaries, three appeared in the animals treated with DMBA. An increase in the tumors in the genitals of exposed animals was not provable.

III.
2 i Brain

Since the evaluation of the changes in the respiratory tracts was uppermost in the exploration, we decided on a histological examination of entire vertical sections of the head. These allow, as ill. 54 shows, for a survey of the mucous membranes of the cavity of the nose as well as the oral cavity, respectively the palate. At the same time, these sections permit an evaluation of the brain areas present on the same level.

Changes in the brain which we designated as calcareous glands occured relatively frequently. It was a case of globular, layered calcium secretions which occured predominantly in the bulbus olfactoris and were generally found in the area around small vessels (ill. 55). These calcium concrements could be the results of an inflammable virus infection according to the opinion of Prof. STOCKDORPH to whom we presented these preparations. Similar changes have also been described in the human brain (ERBSLOH, 1944).

The chart shows the frequency of the calcareous glands in the brains of the individual test groups. Reliable differences in frequency are not provable.

A meningitis or an encephalitis occured only in individual animals proceeding from phlegmons in the head region.